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PRE-APPEAL BRIEF REQUEST FOR REVIEW	Docket Number (Optional) ROSSI=10	
I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to "Mail Stop AF, Commissioner for Patents, P.O. Box 14550, Alexandria, VA 22313-1450" [37 CFR 1.8(a)] on _____ Signature _____ Typed or printed name _____	Application Number 10/586,141	Filed July 2, 2007
	First Named Inventor Mara ROSSI et al.	
	Art Unit 1646	Examiner J. T. Seharaseyon

Applicant requests review of the final rejection in the above-identified application. No amendments are being filed with this request.

This request is being filed with a notice of appeal.

The review is requested for the reason(s) stated on the attached sheet(s).
Note: No more than five (5) pages may be provided.

I am the

- ☐ applicant/inventor
- ☐ assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)
- ☒ Attorney or agent of record. Registration number 37,971.
- ☐ attorney or agent acting under 37 CFR 1.34. Registration number if acting under 37 CFR 1.34 _____

/ACY/	Signature
Allen C. Yun	Typed or Printed Name
202-628-5197	Telephone number
February 14, 2011	Date

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☐ *Total of forms are submitted.

REASONS WHY REVIEW IS REQUESTED

Following the Advisory Action of December 28, 2010, the sole rejection remaining in the instant application is the rejection of claims 1-3 and 5-8 under 35 U.S.C. §103(a) as being unpatentable over Musacchio et al. (1996) in view of Li et al. (WO 98/14467) and Proudfoot et al. (WO 02/28419).

The representative independent claim in Jepson format is claim 1, which reads as follows:

> 1. In a process for the recovery of a chemokine expressed in prokaryotic host cells as inclusion bodies and its subsequent purification, comprising a step of solubilization of aggregated chemokine protein in inclusion bodies/denaturation and a step of renaturation/refolding, the improvement consisting of interposing a Reverse Phase Chromatography step between the step of solubilization of the aggregated proteins in the inclusion bodies/denaturation and the renaturation/refolding step.

As written in Jepson format, applicants concede in the preamble of claim 1 that it is conventional in the art to include the steps of solubilizing/denaturing aggregated chemokine protein in inclusion bodies and renaturing/refolding the chemokine protein in a process for recovery and purification of a chemokine protein expressed in prokaryotic host cells as inclusion bodies. Dependent claims 6 and 7 recite a more detailed order of steps according to two embodiments of the present invention.

What is not conventional and not obvious is the improvement discovered by applicants when a step of Reverse Phase Chromatography (RPC) is interposed between the solubilizing/denaturing of inclusion body step and the renaturing/refolding step. The convention in the art is to renature/refold the protein into its correct native conformation before purifying the protein using conventional protein purification techniques.

In the final Office Action of September 14, 2010, the obviousness rejection is maintained for the reasons set forth in the Office Action of March 26, 2010, where the examiner stated that:

> Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the process of Musacchio et al. to purify chemokine mutant of SEQ ID NO:1 by the

> teachings of Li et al. and Proudfoot et al. with a reasonable expectation of success because Li et al. discloses the purification of chemokines from [sic] E. coli and Proudfoot discloses a mutant of SEQ ID NO: that is expressed in E. coli. The motivation to do so is provided by Proudfoot et al. in its disclosure of the process of chemokine mutant purification.

The error in the examiner's rejection is that one of ordinary skill in the art would not be motivated to interpose an RPC step between a solubilizing/denaturing step and a renaturing/refolding step according to the logic used by the examiner.

First, it should be emphasized that both Li and Proudfoot teach recovering and purifying inclusion bodies of chemokines (specifically RANTES or a RANTES mutant protein in Proudfoot) in the conventional manner. On pages 10-11 of Proudfoot, the solubilized inclusion bodies of RANTES were renatured before proceeding with purification steps such as ion exchange chromatography. The only other mention of RANTES purification is in *Pichia pastoris* where RANTES was expressed and secreted into the culture medium in soluble form (instead of as inclusion bodies needing to be solubilized and renatured/refolded).

Similarly, Li teaches at page 12, lines 4-6 that:

> The recovered, solubilized protein may then be purified using conventional techniques. Optionally, if deemed necessary, the target protein may be refolded prior to purification. (emphasis added)

It is therefore clear from Li's disclosures and teachings that purification steps, such as reverse phase chromatography, would be done immediately after solubilization only if there is no need for refolding. See page 15, lines 1-2, where Li teaches that:

> After solubilization and, optionally, refolding of target proteins, these proteins can be recovered and purified by methods well known in the art.

One of ordinary skill in the art can only interpret this as teaching the order of steps to be either 1) solubilization and 2) purification with no need for refolding necessary or 1) solubilization, 2) refolding and 3) purification. In any event, there is absolutely no teaching in Li that would suggest a purification step, such as reverse phase chromatography (RPC), after solubilization but before the

refolding/renaturation step, as is presently claimed. This order of steps is further confirmed by the process for purifying chemokines from bacterial inclusion bodies that is disclosed at pages 24-28 of the Li reference. Clearly, reading Li, one of ordinary skill in the art would be taught to purify chemokine only after a refolding step, if the refolding step is warranted.

Conventional wisdom is to purify only when a protein is in its native (favored) conformation, i.e., purify only after a denatured protein is renatured and refolded, as specifically taught in Li and Proudfoot. Otherwise, a solubilized but denatured protein would be a heterogeneous mixture of different (predominantly incorrect) conformations, many of which are merely transient. Purifying such a "heterogeneous" mixture, although homogeneous in molecular weight, would be problematic and contrary to conventional wisdom that would seek to obtain a homogeneous renatured protein in its native favored conformation first before starting with purification.

Turning now to Musacchio as the primary reference, it should be pointed out here that Musacchio's teaching is a bit unusual in that purification via RPC was the only purification process leading to success, i.e., the recovery of pure recombinant hybrid Opc protein (see abstract and page 751, right column, where the hybrid protein is a fusion of Opc protein with the N-terminal of the high molecular weight meningococcal protein P64k as a stabilizer) renatured with a conformation suitable enough to generate functional antibodies in mice capable of killing meningococci in the presence of human complement . See also page 755, first paragraph of the left column, where it is taught that "other purification procedures were used without success." One of ordinary skill in the art would readily recognize that Musacchio's interposition of an RPC step is a special unusual case where it offered the only way (after other purification procedures were used without success) to the successful recovery of a hybrid Opc protein, a protein which would also be appreciated as being produced as a fusion protein for purposes of stability. With such an understanding of the unusual circumstances in Musacchio, this same person of ordinary skill in the art would certainly not be motivated to charge against conventional wisdom as discussed above and modify the specific teachings of Li and Proudfoot regarding recovery and purification of a chemokine from inclusion

bodies by interposing the RPC step of Musacchio between the solubilizing/denaturing step and the renaturing/refolding step.

Furthermore, while Musacchio, as an isolated teaching of a special case of a hybrid fusion protein refractory to other purification procedures, discloses interposing an RPC step between the solubilization step and the refolding step for the purification of this hybrid Opc protein fusion, Musacchio also teaches that the order of the refolding and purification (i.e., RPC), at least insofar as they relate to this special case, is not important. See page 757, second full paragraph in the left column, where it is taught that, in preliminary experiments, protein renaturation carried out prior to purification leads to similar results as when pure protein (i.e., renaturation after purification) was used. Even though these results are from preliminary experiments in Musacchio, one of ordinary skill in the art would nevertheless be taught that performing the purification step prior to renaturation does not lead to any improvement. Those of ordinary skill in the art reading Musacchio would believe that the only critical parameter in Musacchio is the use of RPC for the purification step (and that this is specific to the hybrid Opc protein fusion that Musacchio was working with).

By contrast, the presently claimed process , which interposes an RPC step between solubilization and renaturation, leads to an improvement in achieving high yield and purity of recombinant chemokines (see the present specification, page 2, first four lines of the Description of the Invention section and page 18, last four lines).

One of ordinary skill in the art, faced with the specific teachings of Li (and of Proudfoot) that a denatured chemokine (from solubilization of chemokine inclusion bodies) should be purified only after a refolding step (and only if this refolding step is warranted in the overall recovery and purification process) and the further disclosure in Musacchio that performing the RPC step before the renaturing/refolding step does not lead to any improvement, would have absolutely no motivation to act against conventional wisdom and interpose an RPC purification step between solubilization/denaturation and renaturation/refolding. There is no suggestion anywhere of any reason to modify the procedure of Li (as modified by Proudfoot). Thus, not only would this person

of ordinary skill in the art have no such motivation but this same person would indeed also have no expectation that such an interposition contrary to conventional wisdom would lead to an improvement in the yield of purified protein.

For all the reasons discussed above, it is urged that the obviousness rejection be withdrawn following pre-appeal brief review and this application be passed on to allowance.